Turkish Journal of Biology

Volume 23 | Number 2 Article 5

1-1-1999

Anthranilate Synthetase From Escherichia coli SJHI:Purification and Some Properties

MÜNEVVER ARISOY

Tr. J. of Biology
23 (1999) 169-175

Anthranilate Synthetase From *Escherichia coli SJHI:* Purification and Some Properties

Münevver ARISOY
Ankara University, Faculty of Health Education, Keçiören, Ankara-TURKEY

Received: 05.01.1998

Abstract: A procedure employed in the purification of anthranilate synhetase of *Escherichia coli SJHI* is described. The purified anthranilate synthetase appeared to be homogeneous when examined with poliacrylamide gel electrophoresis. Phenly-sepharose CL-4B and Blue dye sepharose were used for purification. A positive correlation was found between purification and ammonium sulfate especially using concentrations greater than 10%. A positive effect 2-Mercapto-ethanol on the enzyme stability was also determined.

Key Words: Purification, Anthranilate synthetase, *Escherichia coli SJHI*.

Antranilat Sentatazın *Escherichia coli SJHI*'dan Saflaştırılması ve Özellikleri

Özet: Bu çalışmada *Escherichia coli SJHI* dan antranilat sentatazın saflaştırılmasına ilişkin bir yöntem verilmiştir. Saflaştırılmış antranilat sentataz poliakrilamid jel elektroforezinde gözlenmiştir. Saflaştırma için Phenyl-sepharose CL-4B ve Blue dye sepharose kullanılmıştır. Amonyum sülfatın özellikle 10% dan büyük konsantrasyonları kullanıldığında saflaştırma ile aralarında pozitif bir ilişki olduğu saptanmıştır. Ayrıca enzimin stabilitesi üzerine 2-Mercapto-ethanol ün de olumlu bir etkisi olduğu gözlenmiştir.

Anahtar Sözcükler: Saflaştırma, antranilat sentataz, *Escherichia coli SJHI.*

Introduction

Trytophan notable for its variety of important metabolic reactions and products was among the first amino acids shown to be nutritionally essential (1). The activity of anthranilate synthetase, the first enzyme in the L-tryptophan terminal pathway (2, 3, 4), has been regulated by L-trytophan in some bacteria (*Escherichia coli SJHI, Aerobacter aerogenes, Bacillus subtilis, Pseudomonas putida, Brevibacterium flavum)*, yeast (*Sacchoromyces cerevisia*) and a fungus (*Neurospora crassa*) (5, 6). Antharanilate synthetase is inhibited by L-tryptophan (7, 8) and the formation of the enzyme is also repressed by L-trytophan (5). Anthranilate synthetase, M.W.

* This research was supported by IECDF (International Economic Cooperation Development Fund) in Taiwan, Republic of Chine
* Bu araştırma IECDF (International Economic Cooperation Development Fund) tarafından desteklenmiştir.

60000, from *Escherichia coli* consists of nonidentical subunits specified by the two genes, *trp E* and *trp D,* of the tryptophan operon (9). It is specified by the D and E genes, in addition to being required for anthranilate formation, catalyzes the subsequent step in tryptophan synthesis and the conversion of anthranilate to N-(5'-phosphoribosyl) anthranilate (10). The other studies on the anthranilate synthetase of *Salmonella typhimurium* revealed essentially the same subunit relationship (11) and studies on the enzyme isolated from *Aerobacter aerogenes* also indicated that anthranilate formation and anthranilate conversion to N-(5'-phosporibosyl) anthranilate were catalyzed by the same enzyme complex (12).

This paper is concerned with the purification and regulatory properties of anthranilate synthetase from *Escherichia coli SJHI.*

Materials and Methods

Bacterial strains and growth medium

Escherichia coli SJHI was used in this study. The culture medium consisted of liquid broth and cultures grown overnight with aeration at 37°C. Then the cultures were transfered to the new medium, and modified according to the method described by Vogel and Bonner (13), with (gr/L.) 3.5 KH_2PO_4, 3.5 $(NH_4)_2SO_4$, 1 $MgSO_4$. $7H_2O$, 5 Yeast extract, 5 casein hydrolysate, 8 glucose and trace elements. The cultures were grown overnight with aeration at 37°C.

Enzyme assay

Anthranilate synthetase activity was assayed according to the method described by Jensen which determines the anthranilate formed using a spectroflorophotometer (14).

Preparation of crude extract

Cells which were centrifuged at 80000 r.p.m. for 20 minutes, were disrupted with a dyno-mill. After disruption they were concentrated by ultra millipore filtration.

Results and Discussion

Stability of anthranilate synthetase

The effects of 0.1 M tris and 1 mM 2-Mercapto ethanol on the stability of the enzyme for 7 days were compared and it was found that 2-Mercapto ethanol had a positive effect on enzyme stability (Table 1).

Table 1. Stability Test of Anthranilate Synthetase.

	Residue (%)	
Time (day)	Tris-HCI+2 Mercapto ethanol	Tris-HCI
0	100.0	100.0
1	93.8	57.9
5	27.6	35.9
7	15.0	7.5

Comparision of different binding buffers for blue dye sepharose

The optimal binding condition of the enzyme on the blue dye sepharose using ammonium sulfate and magnesium sulfate was studied. A positive correlation was found with ammonium sulfate up to 78% binding yield at concentrations gerater than 10% whereas a decrase was observed in the concentration of 10 mM magnesium sulfate (Figure 1).

Elution of blue dye sepharose with triton x-100

Triton x-100 gradient (0-5%) was used to elute the enzyme from the gel. Lower concentrations of triton x-100 are available but higher levels are necessary to regenerate the gel. After 10 fractions, the eluted protein showed optimum activity at an optical density of about 1600 (Figure 2).

Hydrophobic interaction chromatography (H.I.C) on phenyl-sepharose CL-4B of crude extract

Phenyl-sepharose CL-4B was prepared and equilibrated with 0.1 M Tris HCl buffer, pH: 7.8, containing 25% ammonium sulfate and 1 mM 2-Mercapto-ethanol. The culture extract was applied to the column and then washed with the same buffer. The ethylene glycol gradient (0-

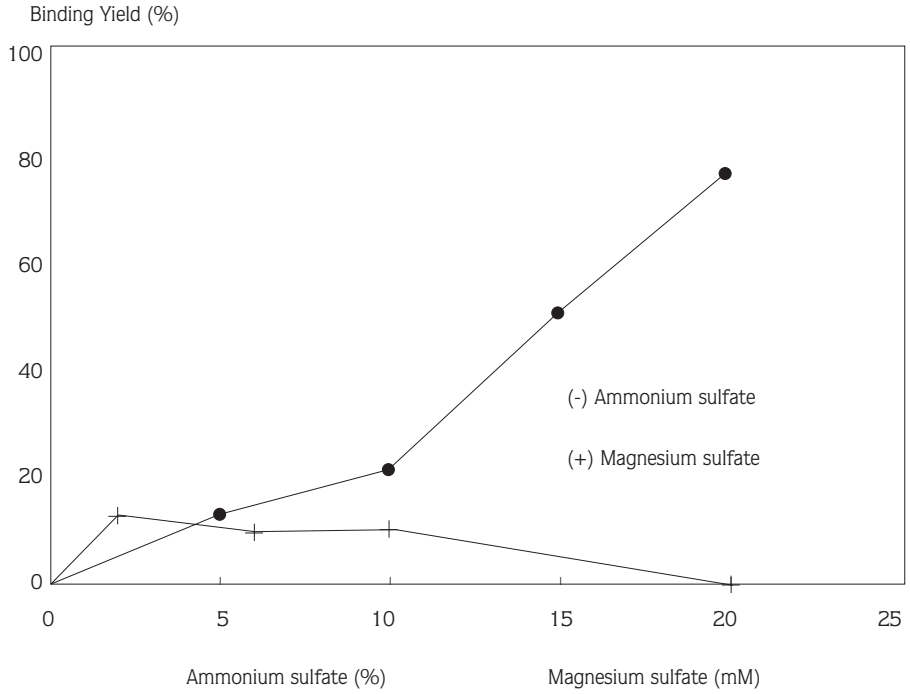

Figure 1. Comparison of Different Binding Buffers for Blue Dye-Sepharose

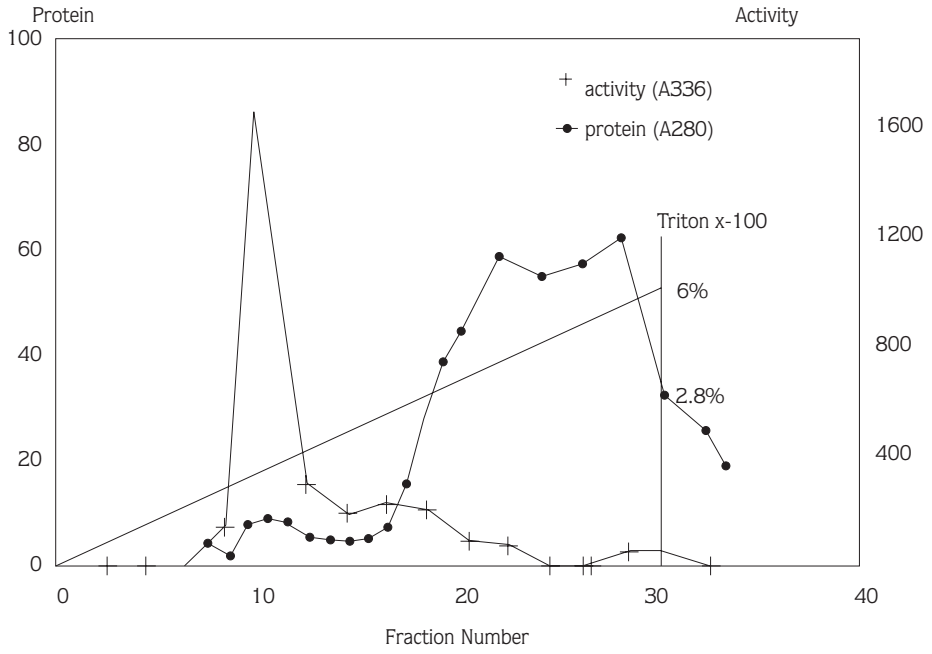

Figure 2. Elution of Blue Dye-Sepharose by Triton x-100

75%) was used for elution. The column flow rate was adjusted to about 25 mL/hr and fractions were collected every 10 minutes. Total protein and anthranilate synthetase activity of each fraction was measured and the fractions which had higher levels of activity were combined to use for blue dye sepharose (Figure 3).

Blue dye sepharose of anthranilate synthetase

Blue dye sepharose was prepared and equilibrated with 0.1 M Tris HCI buffer pH: 7.8 containing 25% ammonium sulfate and 1 mM 2-Mercapto ethanole. The enzyme solution obtained from the H.I.C was applied to the column and then washed with the same buffer. The triton x-100 gradient (0-5%) was used for elution. The flow rate was adjusted to about 25 mL/hr and fractions were collected every 5 minutes. Then the total protein and enzyme activity of each fraction was measured which yielded different maxima for different fractions where optimum activity was obtained after 13 fractions (Figure 4).

Evidence of homogenity

The degree of homogenity of purified anthranilate synhetase was determined by polyacrylamide gel electrophoresis where K indicates the purified enzyme after 10 elutions comprising 2 steps of purification (Figure 5).

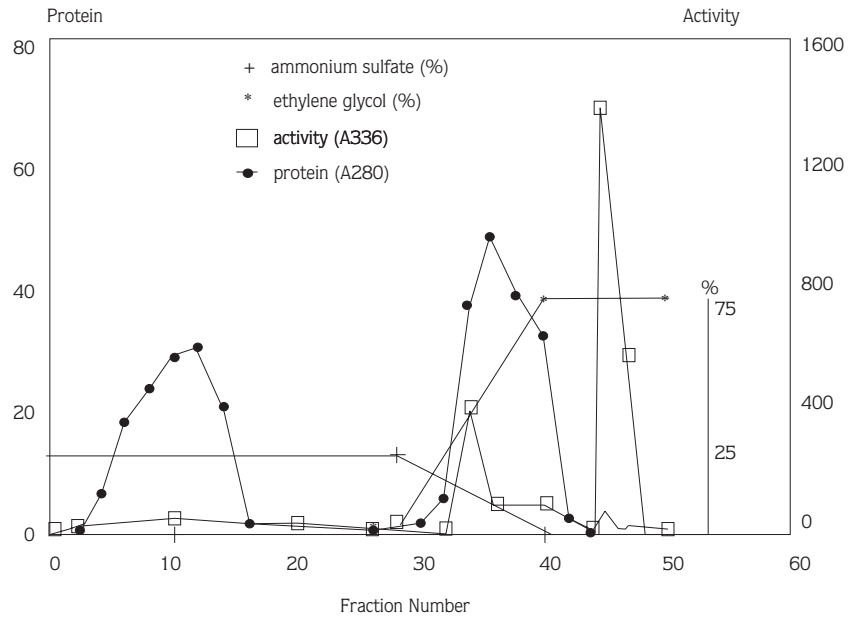

Figure 3. Hydrophobic Interaction Chromatography of Anthranilate Synthetase

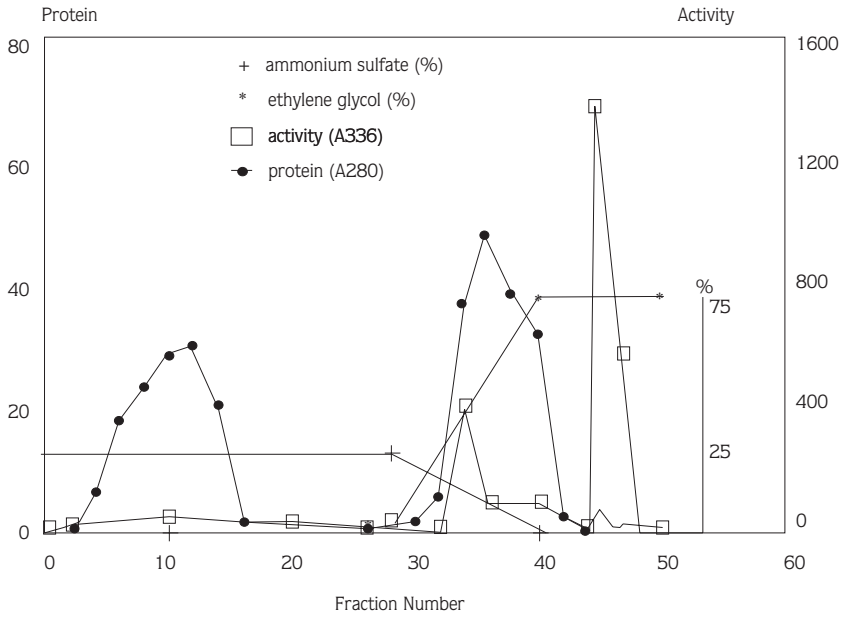

Figure 4. Blue Dye-Sepharose of Anthranilate Synhetase

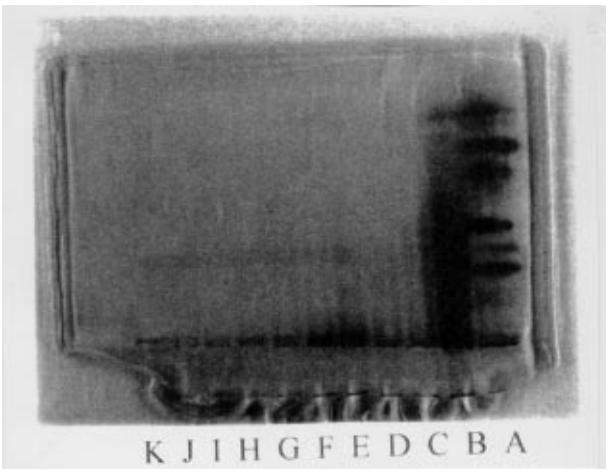

Figure 5. Polyacrylamiyde Gel Electrophoresis of the Purified Enzyme A: The proteins which have low molecular weight B: Crude extract C: After H.I.C. D, e, F, G, H, I, J: After the blue dye sepharose K: The purified enzyme

This technique is quite useful for assessing the purity of enzymes in protein preparations. The procedure proved to be very effective for the removal of inactive proteins. As can be seen from Table 2, the specific activity of anthranilate synthetase increased 32-fold, with only 2 chromatographic steps, with a recovery of 43 % of the total enzymatic activity.

Step	Total protein (mg/mL)	Total activity (U/mL)	Specific activity (U/mg)	Yield (%)	Purification fold (%)
Crude extract	30.15	198000	6567.16	100.0	1.00
H.I.C.	0.43	86280	200651.16	43.58	30.55
Dye-separose	0.40	84500	211250.00	42.67	32.17

Table 2. Purification of Anthranilate Synthetase from *Escherichia coli SJHI.*

References

1. Murray, R.K., Granner, D.K., Mayes, P.A., Rodwell, V.W., 1988, Harper's Biochemistry, 498.
2. Rosaura M. Romero and Margerest F. Roberts, 1996, Anthranilate Synthase From Ailanthus altissima Cell suspension Cultures, Phytochemistry. 41, 2: 395-402.
3. Oswalda, C.S. and Tsune, K., 1991, Molecular Characterization and Expression Analysis of the Anthranilate Synthase Gene of Pseudomonas syringae subsp. savastona, J. Bacteriology. 173, 2: 463-471.
4. Poulsen, C., 1991, High-performance Liquid Chromatographic Assay of Anthranilate Synthase From Plant Cell Cultures, J. Chromatography. 544: 155-160.
5. Hagino, H. and Nakayama, K., 1974, Regulatory Properties of Anthranilate Synthetase from Corynebacterium glutamicum, Agr. Biol. Chem., 39(2), 323-330.

6. Albert L. Lehnınger, 1982, Pathway of Trytophan Synthesis in Escherichia coli, Principles of Biochemistry, Fifth Edition.

7. Hertel, S.C., Hieke, M. and Gröger D., 1991, Anthranilate Synthase from Ruta graveolens. Partial Purification and Properties, Biochem. Physiol. 187: 121-129.

8. Bae, Y. M. and Crawford L. P., 1990, The Rhizobium meliloti trpE (G) Gene Is Regulated by Attenuation, and its Product, Anthranilate Synthase Is Regulated by Feedback Inhibition, J. Bacteriology, 172: 3318-3327.

9. Ito, J. and C. Yanofsky, 1966, The Nature of the Anthranilic Acid Synthetase Complex of Escherichia coli, J. Biol. Chem. 241: 4112-4114.

10. Ito, J., Cox, C. E. and Yanofsky C., 1968, Anthranilate Synthetase and Enzyme Specified by the Trytophan Operon of Escherichia coli: Purification and Characterization of Component I., J. Bacteriology 725-733.

11. Bauerle, R. H., and P. Margolin., 1966, A Mutational Enzyme Complex in the Tryptophan Pathway of Salmonella typhimurium. Comparison of Polarity and Pseudopolarity Mutations, Quant. Biol. 31: 203-214.

12. Egan, A. F., and F. Gibson, 1966, Anthranilate Synhetase and PR-transferase from Aerobacter aerogines as a Protein Aggregate, Biochim. Biophys. Acta 130: 276-277.

13. Vogel, H., and Bonner D. M., 1956, Acetyl-ornithase of Escherichia coli: Partial Purification and Some Properties, J. Biol. Chem. 218: 97-106.

14. Kane, J. F. and Jensen R., 1970, Metabolic Interloc, The Influence of Histidine on Tryptophan Biosynthesis in Bacillus subtilis, J. Biol. Chem. 245: 2384-2390.